

Evaluating persistence in threatened plant translocations: Genetic diversity and mating systems as measures of success

In brief

For the many long-lived threatened plant species targeted for translocation in Australia, measuring success is challenging. New approaches to measuring translocation success include benchmarking genetic variation and patterns of pollination. We applied these methods to translocated and wild populations of five woody shrubs native to south-west Western Australia that have a range of pollinator types.

We found that the genetic diversity of all translocated populations was comparable to or surpassed that of the wild reference populations. Our findings for pollination patterns were, however, mixed, with higher levels of self-pollination and significantly higher inbreeding estimates in both adults and progeny found for two animal-pollinated species. This indicates that while genetic diversity can be maintained or even increased in translocated populations, long-term persistence of some translocated species may be hampered by increased inbreeding and genetic erosion over time if required pollinators are not present in sufficient numbers.

We recommend further monitoring, and that caution be applied when using only demographic and/or genetic diversity measures to assess translocation success of animal-pollinated species.

Background

Many threatened plant species targeted for translocation in Australia are relatively long-lived. The timeframes over which they may reproduce and recruit can provide significant challenges for evaluating translocation success. Flowering and fruiting may take years, or even decades for some species. Translocation success has been measured in many ways, with practitioners commonly using demographic measures such as:

- vegetative growth of individuals
- survival of individuals over time
- survival of individuals to flowering
- presence of reproductive structures
- presence/abundance of seeds
- presence/abundance of a second generation
- capacity to successfully respond to future disturbances such as fire.

No single measure of translocation success will provide certainty to declare a population viable and self-sustaining or provide the foundation for recommending the down-listing or de-listing of a threatened species; rather, a combination of measures will generally be needed.

Traditional approaches using demographic measures remain important and necessary.

However, alternative approaches such as benchmarking genetic variation and patterns of pollination in translocated populations can allow for assessments of long-term persistence and ultimately translocation success that are more timely.

Successful plant translocation requires the establishment of populations with: (1) adequate levels of genetic diversity; and (2) in animal pollinated plants a functional mating system where pollination tends to occur more between different plants, rather than self-pollination.

Evaluating how successfully genetic diversity has been captured in translocations has been recommended for some time, but not widely undertaken. Similarly, assessing patterns of mating (pollination) in animal-pollinated plants that will reflect pollinator presence, abundance and behaviour have generally not been considered when evaluating translocation success. Both genetic variation and patterns of pollination can be readily assessed using DNA-based genetic marker or genomic techniques by comparing genetic diversity and mating systems in translocated populations to wild reference populations.

BELOW LEFT (top left, middle, bottom): *Acacia cochlocarpa*. Image: Andrew Crawford; *Lambertia orbifolia* SRP. Image: Andrew Crawford; *Banksia anaton*a. Image: Andrew Crawford; RIGHT: *Banksia brownii*. Image: Anne Cochrane



Research aims

We aimed to benchmark genetic diversity and mating systems (patterns of pollination) within translocated and wild populations of five long-lived threatened woody shrub species from south-west Western Australia that have a range of pollinator types (see Figure 1). These include:

- Cactus dryandra (*Banksia anaton*a) – primarily bird and mammal-pollinated
- Feather-leaved banksia (*Banksia brownii*) – primarily bird and mammal-pollinated

- Round leaf honeysuckle Narrikup subspecies (*Lambertia orbifolia*) – bird-pollinated
- Round leaf honeysuckle Scott River Plains subspecies (*Lambertia orbifolia*) – bird-pollinated
- Spiral fruited wattle (*Acacia cochlocarpa* subsp. *Cochlocarpa*) – insect-pollinated

Specifically, we wanted to know whether levels of genetic diversity within translocated populations are

equivalent to wild populations, and whether mating systems or patterns of pollination in translocations are equivalent to wild populations. Affirmative answers would provide evidence of translocation success and indicate that sourcing and/or mixing of propagule sources had been effective when establishing translocations, and the development of functioning mating systems and pollinator assemblages.

What we did

We investigated genetic diversity and mating systems in six translocated populations of the four primarily bird/mammal pollinated plants and genetic diversity in three translocated populations of the insect-pollinated spiral fruited wattle, and compared these with wild reference populations.

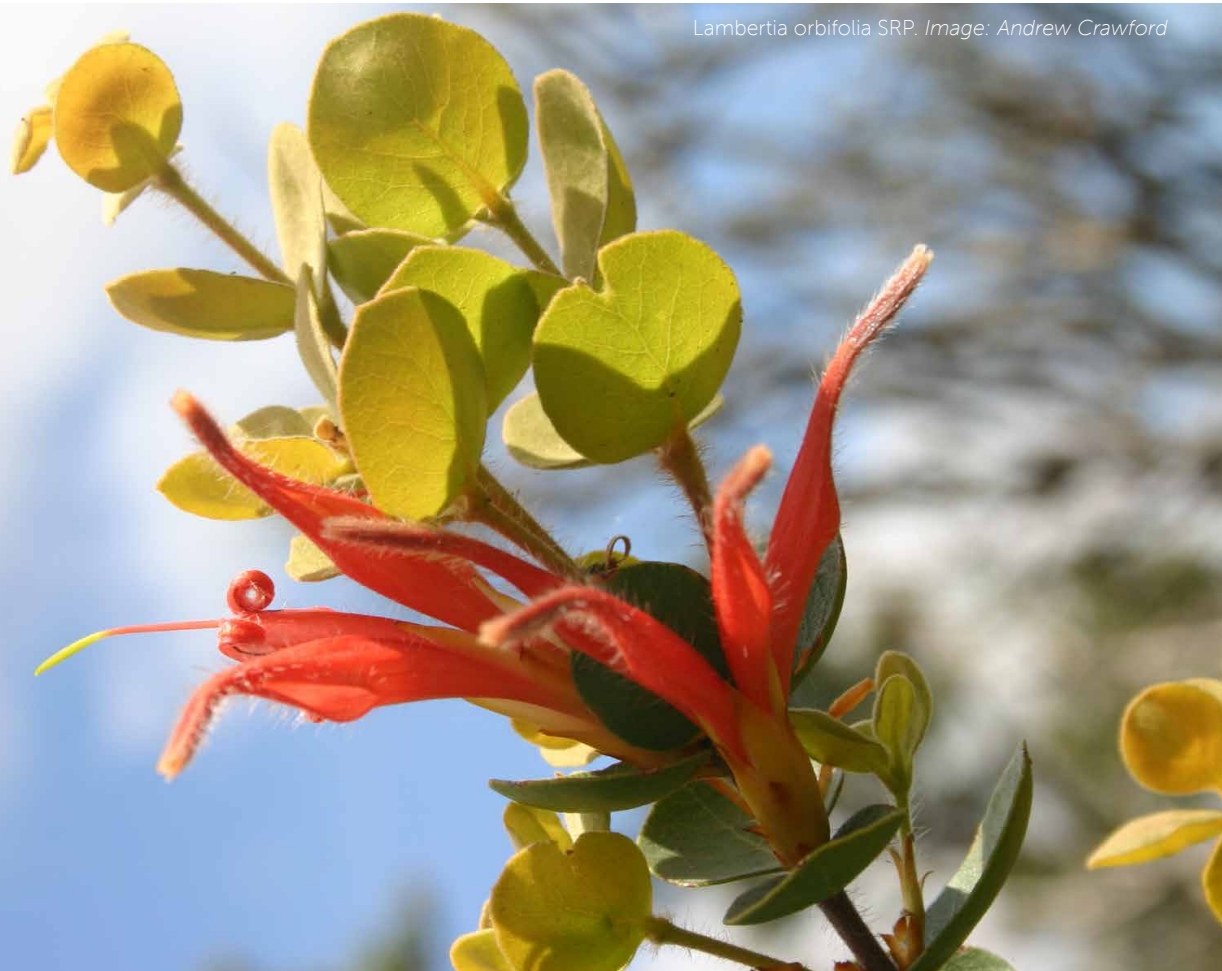
The translocated populations were established in areas in south-west Western Australia with similar soil, habitat and climate to the wild populations, but were outside the current range of each species/subspecies. Significantly, a number of translocations had been established with seed from now-extinct populations. All translocations had been established for at least five years before sampling was carried out from 2012 to 2017.

Scientists and management staff of the Western Australian Department of Biodiversity, Conservation and Attractions (DBCA) and researchers from Murdoch University and The University of Western Australia did the sampling of leaf material and seed from translocations and wild populations. Some seed material that had been collected some years earlier from both wild and translocated populations was stored ex-situ under standard gene-bank conditions in the DBCA Threatened Flora Seed Centre. All seed was germinated and then propagated under nursery conditions for approximately six months at the DBCA Botanic Gardens and Parks Authority nursery. We extracted DNA from both adult leaf material and seedlings. Importantly, once sampled for DNA, seedlings

were used either to augment current translocations or, in the case of cactus dryandra, feather-leaved banksia and both round leaf honeysuckle subspecies, to establish new translocations.

We genotyped (characterised genetically) adult plants and seedling progeny from translocations and wild populations of all five species and subspecies using highly variable DNA markers called Simple Sequence Repeats (SSRs) or microsatellites. These markers allow the accurate assessment of levels and patterns of genetic diversity within and between translocated and wild populations, and also patterns of pollination via paternity analysis (mating system analysis), based on genetic variation in the seedling progeny.

Lambertia orbifolia SRP. Image: Andrew Crawford



Key findings

Genetic assessment of translocated populations of all five plants found that their genetic diversity was comparable to or surpassed that of the wild reference populations.

Two propagule sourcing strategies had been used when establishing the different translocations: taking seed from a single large source population; or mixing seed from two or more usually smaller populations. Our findings indicated that in those cases where the translocation was established from a single source population (spiral fruited wattle, cactus dryandra and the Scott River Plains subspecies of round leaf honeysuckle) the seed sourcing strategies captured adequate genetic diversity. Our results also indicated that the source populations themselves for these plants were genetically diverse and appropriate single sources for seed.

Alternatively, where the translocated population was established by mixing seed from multiple source populations (feather-leaved banksia, Narrikup subspecies of round leaf honeysuckle), genetic diversity was comparable to or higher than that of source populations of known high diversity and, as expected, higher than that of smaller source populations. In this case, strategic mixing of source populations to maximise genetic diversity was seen to be effective. For example, genetic analysis demonstrates that the mixing of two different source populations to establish two translocations in the Narrikup subspecies of round leaf honeysuckle has resulted in genetic representation from both source populations, with an overall increase in genetic variation compared to the individual populations (see Figure 2).

In contrast to our assessment of genetic diversity, which shows a

positive outcome for persistence of all the translocations, our findings for the mating system and patterns of pollination were varied. All four species we investigated had mating systems combining outcrossing (pollination between different plants) and "selfing" (self-pollination) in varied proportions among species and among populations.

The mating system in four of the species is predominantly by bird pollination and, in the case of the two *Banksia* species (cactus dryandra and feather-leaved banksia) also pollination by honey possums and other mammal species, and probably insects. If mating systems were comparable between translocated and wild populations, we could be confident that the translocation is functioning effectively in terms of the mating system and associated pollinator services.

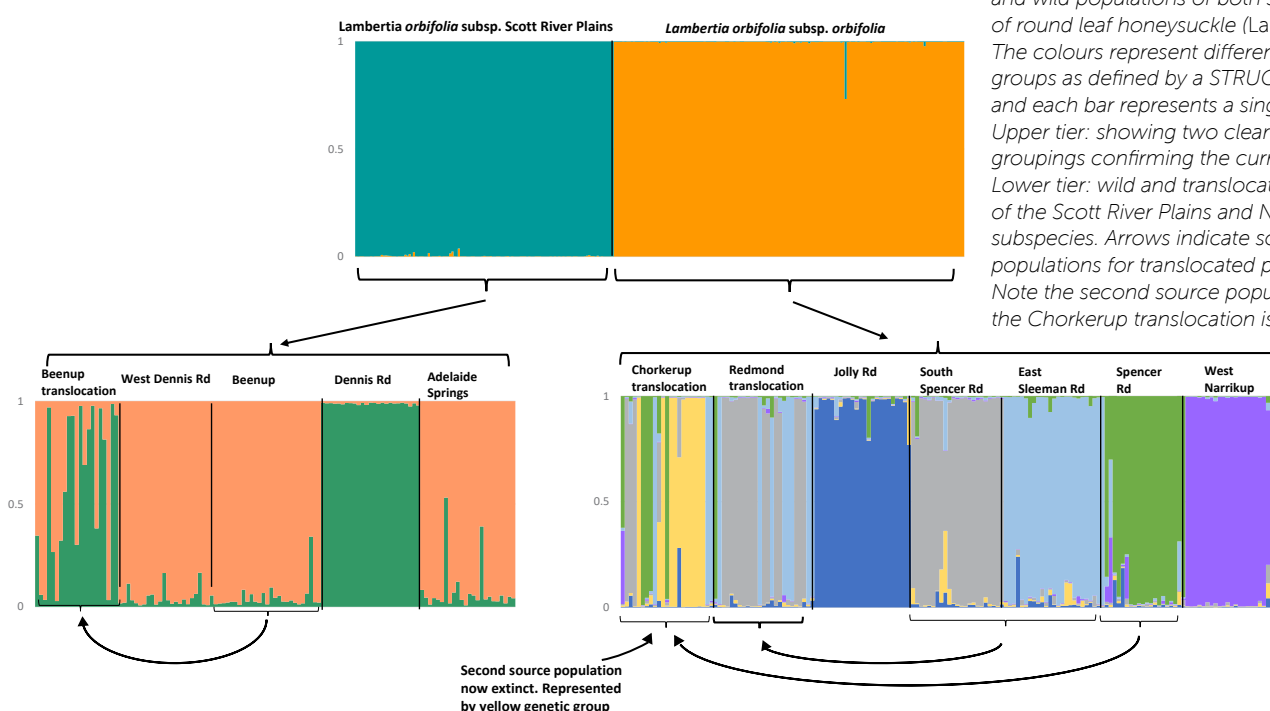


Figure 2. Genetic analysis of all translocated and wild populations of both subspecies of round leaf honeysuckle (*Lambertia orbifolia*). The colours represent different genetic groups as defined by a STRUCTURE analysis, and each bar represents a single plant. Upper tier: showing two clearly distinct groupings confirming the current subspecies. Lower tier: wild and translocated populations of the Scott River Plains and Narrikup subspecies. Arrows indicate source populations for translocated populations. Note the second source population for the Chorkerup translocation is now extinct.

Key findings (continued)

In two species, cactus dryanda and the Narrikup subspecies of round leaf honeysuckle, this was the case. In both, we found that mating system estimates for translocated populations largely reflected those in wild populations.

However, in the feather-leaved banksia and the Scott River Plains subspecies of round leaf honeysuckle, there were higher levels of selfing and significantly higher inbreeding estimates in both adults and progeny at both sites. Although we have no direct observations of pollinators in these translocated populations, the higher levels of selfing suggests that pollinator activity is suboptimal and could ultimately result in reduced seed set or seedling fitness associated with inbreeding.

Interestingly, these translocated populations with higher levels of selfing and significantly higher inbreeding estimates were all

established by the mixing of seed from two or more source populations (see Figure 2). While increased selfing is potentially an ongoing concern, the increased inbreeding estimates may be due to the survival of less-fit (inbred/selfed) individuals during ex-situ propagation of seedlings or may be associated with a genetic sampling effect known to occur in admixed populations known as the Wahlund effect. The effect of the latter would be expected to lessen over time but the survival of inbred individuals during *ex-situ* propagation and their subsequent establishment in the population is of concern. It seems likely that less-fit seedlings survived to adulthood because they were nurtured through the vulnerable seed germination and plant establishment phase in the laboratory and nursery, where selective pressures are likely to be far less than those in the wild. This requires further investigation.

While our findings indicate that genetic diversity has been maintained or even increased in all translocated populations investigated in the five species/subspecies and that seed sourcing strategies have been effective, patterns of mating also need to be considered when evaluating the long-term persistence of animal-pollinated translocated plant populations. Higher levels of inbreeding in some translocated populations are of concern and, although possibly only temporary, require further monitoring. It is also important to note that plant mating systems may vary over both time and space and, as such, these studies represent only a snapshot in time. Nevertheless, if the mating patterns in the translocation populations revealed so far continue on a similar trajectory, there is a likelihood of increased inbreeding and genetic erosion over time.

Acacia cochlocarpa. Image: Andrew Crawford





BELOW: Banksia ananeta seed collections.
Image: Sarah Barrett

Implications and recommendations

Our findings provide an optimistic assessment of translocation success in capturing sufficient genetic diversity in all five plants through careful seed sourcing strategies and/or where strategic mixing of seed sources to maximise genetic variation is implemented.

While common features of threatened plant translocations, such as small population size, genetically limited sources of propagules and novel habitat, can render them vulnerable to negative effects associated with reduced genetic diversity, these effects can be largely negated by appropriate strategies for sourcing and admixing seed and other propagules. Detailed [guidelines](#) are now available that outline seed collection methodologies and strategies for maximising genetic variation in translocated plant populations. Approaches set out in these guidelines were followed when planning the translocations of all five threatened plants investigated in this study, and we have been able to confirm their suitability for creating genetically diverse translocations.

The initial establishment of sufficient genetic diversity is important and likely to be critical

if the translocated populations are to avoid inbreeding effects and to have the genetic resources to adapt to changing environmental conditions. However, there is also the need to avoid longer-term erosion of genetic diversity through unfavourable patterns of mating in animal-pollinated plants, especially in the case of long-lived species. We therefore recommend that caution be applied to such species when using only demographic and/or genetic diversity measures to assess translocation success. Ensuring the presence of pollinators and their abundance – in the case of our examples, birds and mammals – is an important first step. Further, understanding how the pollinators may disperse pollen and influence mating events such as outcrossing and selfing will be important for assessing the risk of undesirable levels of inbreeding and longer-term loss of genetic diversity.

In this study we have highlighted the use of molecular genetic techniques to benchmark genetic diversity and mating system variation in the translocated populations as valuable approaches to assessing translocation success.

While the genetic data we used can be extremely useful in



planning, assessing and predicting translocation success, it is unlikely to be available for most plant translocations in Australia. However, it is possible to ensure adequate levels of genetic diversity in translocated populations by carefully following seed sourcing and seedling establishment protocols and using sufficient numbers of founders, as detailed in current guidelines.

It is also possible to at least indirectly assess effective pollinator performance and mating system outcomes by evaluating seed production, and pollinator abundance and behaviour, in the translocated populations.

Cited material

Monks L, Standish R., McArthur S., Dillon R., Byrne M. and Coates D. Genetic and mating system assessment of translocation success of the long-lived perennial shrub *Lambertia orbifolia* (Proteaceae). *Restoration Ecology* (in press). doi.org/10.1111/rec.13369

Further Information

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